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(71) Applicant *(for all designated States except US)*: CULTOR OYJ (CORPORATION) [FI/FI]; Kyllikinportti 2, P.O. Box 105, FIN-00240 Helsinki (FI).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: MAUGLE, Paul, David [US/US]; 88 Central Avenue, Norwich, CT 06360 (US).

(74) Agent: D'ZURILLA, David; Lane, Aitken & McCann, Suite 901, 2600 Virginia Avenue, N.W., Washington, DC 20037 (US).

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(54) Title: PROCESS OF ENHANCING GROWTH AND SURVIVAL OF AQUATIC ORGANISMS THROUGH WATER BORNE ENRICHMENT WITH STABLE VITAMIN C DERIVATIVES

(57) Abstract

The disclosed invention discloses a process to increase the growth and survivability rates of shrimp and other aquatic organisms, and the hatchability of fish eggs. Stabilized forms of vitamin C are added directly to aqueous media where aquatic organisms develop through the larval stage, and the aquatic organisms absorb the vitamin C derivatives. The aquatic organisms treated in this manner have a greater chance of surviving to maturity in grow-out ponds, resulting in increased production.

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Process of Enhancing Growth and Survival of Aquatic Organisms Through Water Borne Enrichment with Stable Vitamin C Derivatives

Background of the Invention

Field of the Invention

The present invention relates to the enhancement of growth and survival rates of fish, shrimp, and other forms of aquatic life through the addition of stable vitamin C derivatives to aqueous media in which the aquatic organisms are reared.

Description of Related Art

The raising of fish, shrimp and other forms of aquatic life as foodstuffs has become commonplace in many countries. The process of rearing such aquatic life generally involves several steps. For example, shrimp eggs are fertilized and placed into rearing tanks, and the resulting zygotes are allowed to develop in the tanks until the 2 day old Post Larvae stage. Upon reaching the 2 day old Post Larvae stage, the larvae are transferred to a raceway where the shrimp further develop. After reaching the 10 day Post Larvae stage in the raceway, the Post Larvae are transported to a grow-out pond in which the shrimp are allowed to fully mature.

For raising and hatching fish, eggs from several females and sperm from several males are stripped or spawned and then pooled together in separate containers. Portions of sperm are dry mixed with the eggs to a uniform consistency. An

aqueous medium is then added to the fertilized eggs, and the eggs are allowed to harden while immersed in the fertilized mixture for up to one hour. The eggs are incubated in trays until the appearance of eyes--an indication of successful fertilization. The successfully fertilized organisms are transferred to tanks or ponds for development.

One problem encountered when raising aquatic organisms in aquatic environment is the deleterious effect that changes in the aquaculture have on the aquatic organisms. Shrimp are especially sensitive to deviations in their environment. Small variations in temperature, salinity, and nutrient makeup of the aqueous environment during the shrimp larval rearing cycle lead to stress, and cause decreased survivability of the shrimp larvae. The particularly stressful periods in the raising of shrimp include metamorphosis, transportation from rearing tanks and raceways to grow-out ponds, and the acclimation period associated with pond stocking.

It is known in the art of raising fish, shrimp and other aquatic life in aquaculture that the use of nutrient enriched feeds, such as feeds enriched with vitamin C supplements, increases the number of aquatic organisms produced. These supplements normally contain stabilized forms of vitamin C, such as ascorbic acid 2-sulfate, and also contain other additives. The stabilized form of vitamin C is added to the feed, and the enriched feed is added to the medium in which the aquatic organisms are reared. Stabilized forms of vitamin C are used because ascorbic acid breaks down rapidly

in the presence of copper, iron and calcium ions which are normally present in an aqueous environment.

While it is known in the prior art that feeds supplemented with vitamin C will enhance the growth of aquatic life, the prior art does not disclose the addition of stabilized vitamin C directly to an aquaculture media, during larval and juvenile stages of development of aquatic organisms, prior to putting aquatic organisms in grow-out ponds.

Summary of the Invention

The present invention discloses a process whereby stabilized forms of vitamin C are added directly to media in which shrimp, fish, and other forms of aquatic life are reared. The addition of stabilized vitamin C derivatives to culture waters containing shrimp larvae during larval development results in better survival, increased lipid deposition, enhanced resistance to environmental stresses, and greater larval and shrimp production. The addition of stabilized vitamin C to culture waters containing fish eggs during fish egg hardening improves the hatchability of the eggs resulting in increased production of juvenile fish. As will be demonstrated in the examples disclosed herein, the addition of the chemically stabilized vitamin C directly to aquaculture media, referred to as water borne enrichment, increases the survival rate and growth rate of shrimp, and the hatchability of fish eggs. Stabilized forms of vitamin

C that may be used in the process of the present invention include dipotassium ascorbate 2-sulfate, ascorbyl 2-sulfate, ascorbyl 2-monophosphate, ascorbyl 2-polyphosphate, ascorbyl palmitate, ascorbyl acetate, ascorbic 2-glucoside, and encapsulated forms of vitamin C.

For shrimp and other crustaceans, growth only occurs during molting--the shedding of the exoskeleton as the body grows larger. After the exoskeleton is shed, the larvae take up seawater via osmosis. This osmotic intake of seawater allows the shrimp to absorb nutrients present in the seawater such as vitamin C. Vitamin C is linked to collagen formation and to ion exchange regulation in shrimp and other aquatic life. The uptake of vitamin C by the shrimp during the larval stage results in increased levels of vitamin C in the shrimp which leads to higher survival rates and growth.

A two stage hatchery may be employed in the present invention. The first stage involves fertilizing shrimp eggs and rearing the resulting shrimp larvae in a larval rearing tank through the Nauplii, Zoea and Mysis stages and up to 2 day old Post Larvae. To initiate the second stage, the two day old Post Larvae are transferred to raceways where they are reared until approximately 10 day old Post Larvae. The stabilized vitamin C is added to the raceways after the larvae reach the Zoea larval stage, which occurs approximately 2-3 days after fertilization, and in a preferred embodiment is added on a daily basis up to 5 or 6 day old Post Larvae. During the second stage, the seawater in the raceway is exchanged daily and the volume of the

seawater may be increased with each daily water exchange. If the volume of the seawater is increased, the amount of stabilized vitamin C is commensurately increased to maintain the desired concentration. After reaching approximately 10 days Post Larvae, the larvae are transferred from the raceway to a grow-out pond. If a transport truck is used for this transfer, stabilized vitamin C is normally added to the transport truck. The shrimp are allowed to mature in the grow pond which normally takes approximately 100-150 days. No vitamin C derivatives are added to the grow-out pond, since the feed used in the pond contains a vitamin C supplement.

Consequently, it is an object of the invention to increase the survival and growth rates of shrimp and other aquatic organisms, and the hatchability of fish eggs, through the direct addition of stabilized vitamin C derivatives to aquaculture media.

Description of the Preferred Embodiments

The present invention, as it relates to the treatment of shrimp larvae with water borne stabilized vitamin C derivatives, generally involves a first stage of placing fertilized eggs into a tank filled with seawater, and allowing the resulting zygotes to develop in the tank through the Zoea and Mysis stages into 2 day old Post Larvae. The second stage begins upon reaching the 2 day old Post Larvae stage. The larvae are transferred to a raceway, and treated

on a daily basis beginning in the Zoea stage with a concentration of a stabilized vitamin C derivative. The 2 day old Post Larvae are transferred to the raceways at a lower density than is normally practiced in the industry, which reduces the time, from approximately 20 days to approximately 10 days, that the larvae need be kept in the raceway before transfer to a grow-out pond. The treatment with dipotassium ascorbate 2-sulfate beginning in the Zoea stage is continued until approximately 10 days Post Larvae. Upon reaching 10 day Post Larvae, the larvae are transferred to a grow-out pond. If the larvae are transferred to the pond by truck, vitamin C derivative is added to the culture water in the truck.

The preferred vitamin C derivative for use in connection with the present invention is dipotassium ascorbate 2-sulfate (available under the ASTOS trademark from Cultor Food Science, Inc., New York, New York). Dipotassium ascorbate 2-sulfate possesses two unique characteristics--it is both water soluble and water stable. In particular, while it exhibits solubility characteristics similar to that of ascorbic acid (i.e. a water solubility of greater than 50%), the derivatization prevents the degradation of the molecule by cations present in aquaculture media such as those of copper, calcium and iron. Other vitamin C derivatives that may be used include ascorbyl 2-sulfate, ascorbyl 2-monophosphate, ascorbyl 2-polyphosphate, ascorbyl palmitate, ascorbyl acetate, ascorbic 2-glucoside, and encapsulated forms of vitamin C.

The dipotassium ascorbate 2-sulfate is added at a concentration of 30-150 ppm (30-150 grams per metric ton of water) in the hatchery raceways. In most instances, half of the water is exchanged out of the tank each day during rearing, and the concentration of dipotassium ascorbate 2-sulfate is brought back up to the proper concentration (30-150 ppm) after each changeout. Typically a 20 metric ton rearing tank, with a maximum of 15 metric tons of seawater, is stocked with Zoea at a concentration of 80 per liter, and such a setup can result in as many as 1.2 million Post Larvae shrimp.

Upon reaching 10 day old Post Larvae, shrimp from the treated raceways are pooled for transport. During transport 30-150 grams of dipotassium ascorbate 2-sulfate per metric ton are added to the transport tank to maintain a constant 30-150 ppm concentration of dipotassium ascorbate 2-sulfate. After the Post Larvae have acclimated to pond conditions, they are then stocked in nursery or grow-out ponds. Shrimp that has been enriched with water borne dipotassium ascorbate 2-sulfate will have increased tissue levels of ascorbic acid (vitamin C) before maturing in the grow-out pond. The shrimp are allowed to mature in the grow-out ponds for 100-150 days. During the time in the grow-out ponds, the shrimp may be fed vitamin C enriched food supplements.

In addition to the use of vitamin C in shrimp rearing, the addition of stabilized vitamin C at doses of 25-150 ppm during fish egg hardening improves egg hatchability and

survival resulting in the production of increased quantities of juvenile fish.

The following examples, which are meant to be illustrative in nature and not at all limiting, further illustrate the steps of the process, the effects of the process on the survival rate, growth rate, and total growth of treated shrimp and other aquatic organisms, and the benefits of water borne addition of vitamin C as compared with the supplementation of feed with vitamin C. In particular, the examples illustrate that water borne treatment with vitamin C derivatives enhances the survival of post larvae shrimp during hatchery rearing, transport, acclimation and subsequent grow-out of Penaeus vannamei.

Example I

Water Borne Dose Response Evaluation Using Penaeid Shrimp

Penaeus vannamei nauplii from a single gravid female were placed into 280-liter conical tanks at a concentration of 100 nauplii per liter. A first treatment of either dipotassium ascorbate 2-sulfate or ascorbyl 2-polyphosphate was added on the second day of the Zoea life stage, and continued each day until the third day of the Mysis stage. The exact dosages of the vitamin C derivatives are outlined in Table 1. Each dosage was replicated in four tanks, and the control was replicated in three tanks.

The dipotassium ascorbate 2-sulfate was dissolved in seawater, and directly added to the rearing tanks. Ascorbyl

2-polyphosphate is not as soluble as the dipotassium ascorbate 2-sulfate, and it therefore had to be mixed with seawater in a blender for five minutes prior to further dilution and addition to the shrimp rearing tanks.

During the Mysis stage, water was exchanged each day by reducing the water in the tank by 90% (while maintaining the larvae in the remaining 10% of the water), and then adding fresh seawater. The larvae were retained in the tank during this short time period with a fine mesh netting material. The correct vitamin concentrations were maintained by adding the required amount of the appropriate ascorbic acid derivative after each water exchange.

The larvae in the tanks were reared through day 9, and the percentage of surviving larvae for each tank was calculated. These results are summarized in Table I. As can be seen from Table I, the tanks treated with dipotassium ascorbate 2-sulfate yielded significantly higher percentages of surviving larvae than either the tanks treated with the ascorbyl 2-polyphosphate or the control. The tanks treated with 50 ppm of the dipotassium ascorbate 2-sulfate had a 70% survival rate through third stage Mysis. This is nearly three fold the survival rate of the controls and more than double the survival rate in the tanks treated with ascorbyl 2-polyphosphate. Although not noted in Table I, the larvae enriched with dipotassium ascorbate 2-sulfate at any concentration in the 50-150 ppm range completed development to third stage Mysis earlier than did the control tanks or the tanks treated with ascorbyl 2-polyphosphate. The tanks

treated with 50 ppm of the dipotassium ascorbate 2-sulfate, in addition to showing the greatest survival rate, showed the earliest development to third stage Mysis.

Table I

Percentage of Larvae Developing into Third Stage Mysis at Day 9

Dosage*	Equivalent ascorbic acid level	Percent surviving larvae	Standard deviation
None	0	23.8	9.6
50 ppm A2S	24 ppm	70.0	3.5
100 ppm A2S	48 ppm	55.3	14.9
150 ppm A2S	72 ppm	48.8	7.4
300 ppm A2PP	75 ppm	30.5	12.6

* A2S = Dipotassium ascorbate 2-sulfate
A2PP = Ascorbyl 2-polyphosphate

Example II

Water Borne Dose Response Evaluation Using Penaeid Shrimp

Penaeus vannamei nauplii from a single gravid female were stocked in seawater at a density of 80 nauplii per liter and reared in twelve 500-liter conical tanks. On a daily basis, beginning on the first day of the Zoea life stage and ending on the fifth day of the Post Larvae stage, the rearing waters were enriched with the dosages of ascorbic acid derivatives noted in Table II. These dosages were maintained throughout the duration of the test. Each dosage and the untreated control were replicated in three tanks.

Each dosing with the ascorbic acid derivative was added directly to a tank containing 200 liters of seawater. The dipotassium ascorbate 2-sulfate dissolved quickly, while the ascorbyl 2-polyphosphate did not completely dissolve. The volumes of seawater in the tanks were increased to 300 liters on the second day and 400 liters on the third day. Beginning on the fourth day, when the majority of larvae had reached Zoea stage 2, 50% of the water was exchanged each day. During the increase in water volume to 300 and 400 liters, and during the water exchange, appropriate amounts of the vitamin C derivative were added to maintain each tank at its proper concentration.

As can be seen from Table II, treatment with 75 ppm of the dipotassium ascorbate 2-sulfate resulted in both the highest survival rate of 79% and also the largest average Post Larvae size. Treatment with 50 ppm of dipotassium ascorbate 2-sulfate, while its survival rate and Post Larvae size were not quite as high as the 75 ppm treatment, did develop somewhat faster than the Post Larvae in the 75 ppm treatment. Table II further shows that ascorbyl 2-polyphosphate decreased survival rate to 6% and slowed development relative to the controls.

Table II

Effectiveness of Treatments

		After 5 days enrichment		After 12 days enrichment		
Dosage[a]	Equivalent ascorbic acid level	Stage of Develop-ment	Average percent survival	Stage of Develop-ment[b]	Average percent survival	Average PL size,[b] mm
None	0	Zoea 3	86	Mysis 2,3, PL 1	13	3.58
50 ppm A2S	24 ppm	Zoea 3	100	Mysis 3, PL 1, 2	69	4.21
75 ppm A2S	36 ppm	Zoea 2	100	Mysis 3, PL 1, 2	79	4.48
200 ppm A2PP	50 ppm	Zoea 2	24	Mysis 2,3	6	

a A2S = Dipotassium salt of ascorbyl 2-sulfate
A2PP = Ascorbyl 2-polyphosphate
b PL = Post-Larval

Example III

Water Borne Dose Response Evaluation Using Penaeid Shrimp

Penaeus vannamei nauplii from a single gravid female were stocked in seawater so that the density at full dilution would be 80 nauplii per liter. The larvae were reared in twelve 500-liter conical tanks. Beginning with the first day of the Zoea life stage and continuing through and ending with the fifth day of the post larvae stage, the rearing waters were enriched with daily dosages of ascorbic acid derivatives as outlined in Table III. Each vitamin dosage level and the untreated control were replicated in three tanks.

This example was initiated with 200 liters of seawater in each tank. On the second day, larvae varied in development stage from Nauplii 5 to Zoea 1, and the water volume was increased to 300 liters in each tank. On the fourth day, after the majority of larvae had reached Zoea 2, the water volume was increased to 400 liters. Also, 50% of the water was exchanged each day from the fourth day to the ninth day. Constant vitamin concentrations were maintained by adding the required amount of dipotassium ascorbate 2-sulfate after each water exchange.

The results in Table III illustrate that the dosage of 50 ppm produced a high survival rate and growth rate at 9 days, while the concentrations of 75 and 100 ppm produced larvae which were slightly more advanced in development (day 2 Post Larvae).

Table III

Yield Data After 9 Days

Dosage[a]	Equivalent ascorbic acid level	Percent surviving larvae	Develop-ment Stage[b]	Average weight, mg	Total biomass, g
None	0	22.1	PL 1	3.71	26.2
50 ppm A2S	24 ppm	36.9	PL 1	3.76	44.5
75 ppm A2S	36 ppm	32.1	PL 1,2	3.50	35.8
100 ppm A2S	48 ppm	27.2	PL 1,2	3.16	27.5

a A2S = Dipotassium ascorbate 2-sulfate
b PL = Post-Larval

Example IV

Effects of Treatment in Hatchery on Shrimp Yield

In a hatchery, commercial scale quantities of shrimp were treated with 30 ppm of dipotassium ascorbate 2-sulfate during the Zoea through Mysis stages, then treated with 50 ppm of dipotassium ascorbate 2-sulfate through Post Larvae stage 7. The post larval shrimp were transferred to separate brackish water ponds to complete development to harvestable size. During the pond rearing period, both the treated groups and the control groups were fed the same commercial feed.

Growth and survival data in both the hatchery and ponds are summarized in Table IV. In the hatchery, treatment with dipotassium ascorbate 2-sulfate gave an improved post larval survival rate averaging 93%, whereas the survival rate of untreated controls averaged 88%. The survival rate in the pond for the treated shrimp was also much better than the untreated shrimp with a 30% average survival rate for the treated shrimp and a 24.4% average survival rate for the untreated. On average, the size, yield, days to harvest, and feed conversion ratios (pounds of feed required to produce one pound of shrimp) were substantially better for the shrimp which had been treated with dipotassium ascorbate 2-sulfate during the hatchery stage than for untreated controls.

A further observation, not noted in Table IV, was a substantial improvement in average weekly growth rate. The growth rate for the shrimp treated with the dipotassium ascorbate 2-sulfate averaged approximately 0.80 grams per week (average weight at harvest/(days to harvest/7)), and for the untreated shrimp averaged approximately 0.65 grams per week.

Table IV

Effects of Treatment on Shrimp Yield and Feed Conversion

Treatment[a]	Percent survival in hatchery	Percent survival in pond	Days to harvest	Average weight at harvest, g	Yield lb/ hectare	Feed Conver-sion Ratio
None	85	19.1	143	12.63	864	2.35
None	91	29.6	156	15.1	1537	2.74
A2S	88	26.1	127	16.06	1496	1.68
A2S	95	26.2	159	16.4	1552	2.2
A2S	95	36.9	130	12.2	1659	1.8
A2S	95	30.8	118	14.8	1504	1.6

a A2S = dipotassium ascorbyl 2-sulfate

Example V

Use of Brine Shrimp Enriched with Ascorbyl 2-Sulfate as Food for Rearing Penaeid Shrimp

Artemia (brine shrimp) nauplii were harvested 21 hours after hatching, transferred to 800-liter tanks, and fed hourly by addition of algae (*Cheatoceros*). Seven hours after initiation of feeding, dipotassium ascorbate 2-sulfate was added at the level of 500 ppm (equivalent to 240 ppm of

ascorbic acid). After 11 and 17 hours, emulsified krill oil was added. The vitamin C enriched brine shrimp were harvested at the "instar II" stage 20-24 hours after initiation of algae feeding and fed directly to *Penaeid* shrimp larvae in stages Mysis 3 to Post Larvae 10.

Two species of *Penaeid* shrimp were raised in three commercial scale trials as described above. Control shrimp were raised in the same way, but with brine shrimp which had not been enriched with dipotassium ascorbate 2-sulfate. Survival data at harvest are summarized in Table V.

Table V

Survival Data for *Penaeid* Shrimp Raised on Treated Brine Shrimp

	Percent survival at harvest	
Penaeid species	Control	Treated
Vannamei	71	79
Vannamei	65	74
Stylirostris	71	80

Example VI

Treatment of Fertilized Salmon Eggs with Dipotassium Ascorbate 2-Sulfate

Eggs from several female salmon are mixed with sperm from several male salmon. An aqueous medium containing 50

ppm of dipotassium ascorbate 2-sulfate is added, and the eggs are allowed to harden for one hour. The fertilized eggs are transferred to trays and incubated. The percentage of eggs which develop into fertilized eggs, as evidenced by the development of eyes, is greater than the percentage which develop from eggs not treated with dipotassium ascorbate 2-sulfate.

CLAIMS

1. A process for enhancing the growth and survival of aquatic organisms raised in an aquaculture media, comprising the steps of:

fertilizing eggs of said aquatic organisms in said aquaculture media, whereby said fertilized eggs transform through Zoea, Mysis and Larvae stages;

adding an amount of stabilized vitamin C derivative to said aquaculture media, whereby said larvae osmotically ingest said vitamin C derivative;

transferring said aquatic organism in said larvae stage to a grow-out pond; and

transferring said larvae to a stocking pond for said larvae to mature.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01K61/00 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01K A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
11 May 1998	20/05/1998

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 215 767 A (MASAKAZU MITSUHASHI) 1 June 1993 see example 4	1
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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P,A	EP 0 819 435 A (LEUVEN K U RES & DEV) 21 January 1998 see column 1, line 41 - line 58 see column 3, line 35 - line 58 see claims 6,7,13,15,17	1

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